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Association between exposure to pyrethroids and chlorpyrifos at age 5 years and IQ at age 7 years among children from the Odense Child Cohort, a prospective birth cohort study

Stine Søgaard Normann[a,*], Helle Raun Andersen[a], Lars Christian Lund[a], Iben Have Beck[a], Flemming Nielsen[a], Niels Bilenberg[b], Christel Nielsen[a,c], þórhallur Ingi Halldórsson[d,e,f], Tina Kold Jensen[a,g,h]

[a] *Department of Clinical Pharmacology, Pharmacy and Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense, Denmark*
[b] *Research Unit of Child and Adolescent Psychiatry, Mental Health Services in the Region of Southern Denmark, University of Southern Denmark, Odense, Denmark*
[c] *Department of Laboratory Medicine, Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden*
[d] *Faculty of Food Science and Nutrition, School of Health Sciences, University of Iceland, 101, Reykjavik, Iceland*
[e] *Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, 2100, Copenhagen, Denmark*
[f] *Molecular Diagnostics and Clinical Research Unit at the Institute of Regional Health Research at University of Southern Denmark, Esbjerg, Denmark*
[g] *Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark*
[h] *OPEN Patient Data Explorative Network, Odense, Denmark*

ABSTRACT

Background: Over the past decade, the use of organophosphate insecticides including chlorpyrifos has faced increasing restrictions due to health concerns, leading to a rise in use of pyrethroids. Concerns about neurodevelopmental insults following pyrethroids exposure exist, but few studies have examined the long-term effects of childhood exposure to chlorpyrifos and pyrethroids on IQ.

Objective: To investigate the prospective associations between pyrethroids and chlorpyrifos exposure at age 5 years and IQ scores assessed at age 7.

Materials and methods: A total of 1083 children from the Odense Child Cohort (OCC), born between 2010 and 2012, were included. Chlorpyrifos metabolite chlorpyrifos-methyl, 3,5,6-trichloro-2-pyridinol (TCPy) and pyrethroid metabolite 3-phenoxy-benzoic acid (3-PBA) were measured in urine at age 5. An abbreviated version of the Danish Wechsler Intelligence Scale for Children fifth edition (WISC-V) was administered at age 7 years.

Results: Median urine concentrations of 3-PBA and TCPy at age 5 were 0.18 μg/L and 1.15 μg/L, respectively. Higher childhood urine TCPy concentration was associated with a reduction in IQ at 7 years (−0.80 (95%CI: −1.29, −0.31)) for each doubling of TCPy. The association was more pronounced in girls (−1.09 (95%CI: −1.80, −0.38)) than in boys (−0.54 (95%CI: −1.21, −0.14)). No association was observed for 3-PBA.

Conclusions: Even in this low exposed cohort, early childhood exposure to chlorpyrifos was associated with lower IQ at age 7. Our results align with previous reports in both animals and humans suggesting that chlorpyrifos exposure may adversely affect neurodevelopment. No association between pyrethroid exposure (3-PBA) and IQ scores was found. The lack of association for 3-PBA is likely due to the low and uniform exposure levels among the participants. To establish a definitive exposure-response relationship, studies or combined datasets with greater variability in exposure levels are required. Continued monitoring and regulation of insecticide use are recommended to protect the health of children.

1. Introduction

Insecticides, particularly organophosphates and pyrethroids, are widely used for crop protection (Zhang et al., 2018). Due to concerns of genotoxicity and suspected developmental neurotoxicity, organophosphate use has been increasingly restricted, with chlorpyrifos banned in the EU in 2020 (European Food Safety, 2019). Consequently, use of the substitutes pyrethroids have increased (Zhang et al., 2018). Despite these regulatory measures, recent European human biomonitoring studies still detect the chlorpyrifos metabolite TCPy in urine, likely from imported foods (Andersen et al., 2022; Norén et al., 2020).

The primary route of exposure to insecticides in the general population is through food residues (Baudry et al., 2019; Glorennec et al., 2017; Morgan, 2012). Following absorption, these compounds undergo rapid metabolism and are predominantly excreted in urine within a few days, making urinary metabolite concentration a valuable biomarker of

* Corresponding author. University of Southern Denmark, Department of Public Health, Clinical Pharmacology, Pharmacy and Environmental Medicine Campusvej 55, Toften, DK-5000, Odense C, Denmark.

E-mail address: snormann@health.sdu.dk (S.S. Normann).

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current exposures (Andersen et al., 2022; Llop et al., 2017).

Both organophosphates and pyrethroids target the nervous system of insects (Abreu-Villaca and Levin, 2017). Organophosphates primarily act by inhibiting acetylcholinesterase (Sultatos, 1994), while pyrethroids primarily inhibit voltage-gated sodium channels (Soderlund, 2020). Therefore, both insecticides possess neurotoxic properties that may also affect mammals, including humans (Abreu-Villaca and Levin, 2017; Sultatos, 1994). It is well known that exposure during vulnerable windows of brain development may have a long-term impact on neurodevelopment (Abreu-Villaca and Levin, 2017; Grandjean and Landrigan, 2014). Although pyrethroids have lower acute mammalian toxicity compared to organophosphates, animal models have demonstrated long-lasting alterations in brain function following gestational or early-life exposure to pyrethroids (Abreu-Villaca and Levin, 2017; Pitzer et al., 2021). Additionally, vulnerability to neurodevelopmental disturbances from insecticides may manifest in a sex-specific manner due to sex-differences in hormones, gene expression and developmental timelines (Comfort and Re, 2017).

In a previous study from the Odense Child Cohort, we observed no association between prenatal insecticides exposure and IQ at age 7 years (Normann et al., 2024). However, the most sensitive exposure window remains to be clarified, and as neurodevelopment continues for several years after birth, exposure during childhood may still be associated with neurodevelopmental insults. Recent studies have also examined associations between exposure to insecticides in childhood with neurodevelopmental outcomes, but the results have been inconsistent (Andersen et al., 2022; Elser et al., 2022; Gonzalez-Alzaga et al., 2014). One limitation of these studies has been the use of surrogate measures of exposure and only a few of these studies used biological samples to quantify exposure (Ntantu Nkinsa et al., 2023; Wang et al., 2016). A study in Nanjing, including 406 children from three kindergartens (both urban and rural areas), reported a significant IQ decrease in highly exposed children aged 3–6 years, with both exposure to chlorpyrifos and pyrethroids and the IQ outcome measured the same day (Wang et al., 2016). Also, the Canadian MIREC-CD Plus study observed a negative cross-sectional impact on IQ from dietary pyrethroid exposure with a mean at 0.39 μg/L in 179 children aged 3–4 years, especially in girls (Ntantu Nkinsa et al., 2023). These findings highlight the need for further research in children with low exposure levels.

This study seeks to address these limitations with the use of longitudinal data and analyses of gender differences in neurotoxic effects at lower exposure levels. We investigated associations between exposure to pyrethroids and chlorpyrifos by measuring exposure biomarkers in urine collected at age 5 years and IQ at age 7 in children from a prospective birth cohort.

2. Methods

2.1. Study population

We used data from the prospective Odense Child Cohort study (OCC), recruitment process has been descried in detail elsewhere (Kyhl et al., 2015). In short, between January 2010 and December 2012, 2874 out of 6707 newly pregnant women (43%) residing in Odense were recruited. After 374 dropouts, the study population consisted of 2500 eligible families, including 52 twin pairs and 2448 singletons. All parents have received oral and written information about the project and have given written consent for their participation. Among the 2448-singleton mother/child-pairs in the Odense Child Cohort a total of 1244 child spot urine samples were collected at 5 years of age and 1510 children completed the IQ test at age 7. Of these, 1092 children had both urine measurements and IQ tests. After excluding 9 cases with missing maternal education level, the final included population of children with both urine samples and IQ-score consisted of 1083 individuals, including 616 boys and 467 girls (Fig. 1), with 42 born preterm.

2.2. Information collected on study participants

During pregnancy, parents completed two questionnaires covering general health, social factors, and lifestyle. Information on birth outcomes and maternal characteristics were also extracted from hospital birth records, including parity, maternal smoking, date of birth, gestational age, child sex, birth weight, length, and head circumference. Data on duration of breastfeeding was collected from self-administered questionnaires completed when the children were 3 and 18 months old, as well as through a sub-project where information on breastfeeding was continuously reported via text messages (Bruun et al., 2016).

Information on the dietary habits of the children at age 7 were collected though questionnaires based on parental report. Parents were asked how often they choose the organic alternative (never/almost never, less than half of the time, about half of the time, more than half of the time, always/almost always, the child does not eat this type of food) for the following food items: fruit/vegetables, milk, cheese, butter, yoghurt, eggs, bread, beef, pork and poultry. We focused explicitly on their responses regarding fruit and vegetables, as intake of non-organic fruit and vegetables are the main source of insecticides in the general population (Baudry et al., 2019). We categorized intake of organic fruit and vegetables into three groups: Almost never organic, half organic and mostly organic intake of fruit and vegetables. If parents provided responses in two nearby categories, we selected the category with the highest organic intake. However, if parents responded 'never organic' for one category but 'always organic' for the other, they were excluded

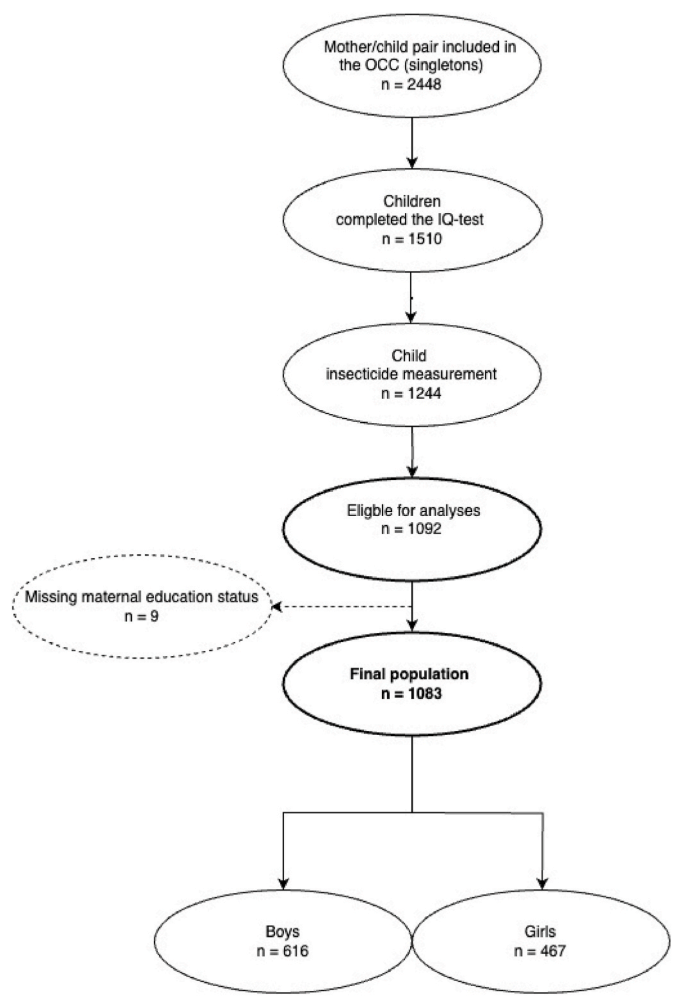

Fig. 1. Flowchart showing selection of the study sample of 1083 mother/child pairs from Odense Child Cohort.

from the group classification. Based on these criteria, 24 participants were excluded.

2.3. Pyrethroid and chlorpyrifos insecticide exposure measurements

The spot urine samples were collected at age 5 years. Pyrethroid and chlorpyrifos insecticide exposure measurements were conducted by analysing the child urine samples for the specific metabolite: TCPy (3,5,6-trichloro-2-pyridinol) representing chlorpyrifos/chlorpyrifos-methyl exposure, and the generic pyrethroid metabolite 3-PBA (3-phenoxy-benzoic acid) representing the combined exposure to most pyrethroids. Although only one measurement has been taken, previous studies have shown that one measurement is representative of exposure (Morgan et al., 2016; Wielgomas, 2013), as seen in previous studies (Andersen et al., 2021; Normann et al., 2024, 2025).

The analyses were performed using high-performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) following the addition of isotope-labelled internal analogues of the compounds as internal standards, deglucuronidation, and solid-phase extraction, as previously described (Dalsager et al., 2019). The accuracy of the analysis was ensured by participation in the German External Quality Assessment Scheme (G-EQUAS) with recovery rates for 3-PBA ranging from 92.7 to 103.3%. The limits of detection (LOD) for the compounds were as follows: 0.30 ng/mL for TCPy and 0.03 ng/mL for 3-PBA.

The creatinine concentrations in urine samples was determined to facilitate adjustment for urine dilution. These analyses were conducted spectrophotometrically using a Konelab 20 Clinical Chemistry Analyzer and a commercial kit from Thermo Fisher Scientific, Vantaa, Finland. Regular participation in the G-EQUAS program ensured the accuracy of the analysis, with excess specimens from this program also included in each sample series. The between-batch variation (CV%) for this analysis was less than 7.0%, and the accuracy ranged from 99.7 to 108.7%.

2.4. Child cognitive assessment

Children were invited to participate in a cognitive function assessment at their school two weeks prior to their 7th birthday. The assessment was conducted by use of an abbreviated version of the validated Danish Wechsler Intelligence Scale for Children, Fifth Edition (WISC-V) (Wechsler, 2014, 2017a). The WISC-V is a widely recognized and validated tool for estimating IQ in school-age children (Wechsler, 2017b). This test comprises four subtests on vocabulary, similarities, block design, and matrix reasoning (Beck et al., 2022), which is commonly used in clinical and research settings (Olivier et al., 2017). The age of 7 years is particularly suitable for IQ assessment, as most major brain functions are accessible for clinical testing, and children are capable of following instructions (Reed and Warner-Rogers, 2011). Four trained psychologists administered the assessments, with the tests primarily conducted by one psychologist, assisted by three others throughout the period, under the supervision of an experienced psychologist. All scoring discrepancies were discussed and resolved by consensus to ensure high interrater reliability (Beck et al., 2023).

From the four WISC-V subtests, a Full-Scale IQ (IQ) was estimated. Additionally, the abbreviated WISC-V test allowed for the estimation of a Verbal Comprehension Index (VCI) based on the vocabulary and similarities subtests. The IQ and Verbal Comprehension Index were standardized to a normal distribution with a mean of 100 and a standard deviation (SD) of 15 using age-appropriate data from the Danish background population (Wechsler, 2017b).

2.5. Statistics

Based on the information available for the full Odense Child Cohort we assessed the possible influence of participant selection by performing a drop-out analysis, comparing the characteristics of those included and not-included into our study (Suppl. Table 1).

For urinary insecticide metabolite concentrations values below LOD were substituted by the metabolite specific LOD/$\sqrt{2}$. Volume-based urinary concentrations (μg/L) were reported as medians along with the 25–75 percentiles. The estimates were transformed to a log2 scale to represent the change in IQ points per doubling of insecticide metabolite concentrations. IQ-scores were normally distributed and reported as mean and SD.

A priori, variables considered to be associated with insecticide exposure and/or child neurodevelopment were selected based on the literature (Andersen et al., 2022; Elser et al., 2022; Gonzalez-Alzaga et al., 2014; Munoz-Quezada et al., 2013). To identify potential confounders, we employed a directed acyclic graph (DAG). We subsequently categorized variables as detailed in Table 1 and examined the variations in maternal urinary 3-PBA and TCPy concentrations, as well as child IQ, according to these maternal and child characteristics.

Predicted changes in IQ on the original scales were plotted for the range of measured insecticides concentrations overall and stratified for sex. To evaluate whether the sex-stratified models were compatible, p-values for the change in IQ were calculated as the difference between coefficients for log-insecticides divided by the pooled variance. Based on the obtain values (z-values) we obtain the p-value from the normal distribution (Fig. 2).

The associations between log2-transformed child insecticide metabolite concentrations at age 5 years and IQ and Verbal Comprehension Index at age 7 years were investigated using multivariable linear regression models for all subjects and stratified by sex (Table 2). All regression models were adjusted for maternal educational level, child sex and urinary creatinine concentrations (measured in g/L).

Table 1
Child median (25th-75th percentiles) urinary concentrations (μg/L) of 3-PBA and TCPy and mean (standard deviation (SD)) Intelligence Quotient (IQ) score according to maternal and child characteristics among 1083 mother-child-pairs from the Odense Child Cohort, Denmark.

All	Total	3-PBA	TCPy	IQ
	N	Median (p25-p75)	Median (p25-p75)	Mean (SD)
	1083	0.18 (0.08–0.36)	1.15 (0.58–2.22)	100 (12)
Maternal education	Maternal characteristics			
High school or less	170	0.16 (0.08–0.38)	1.21 (0.60–2.28)	97 (13)
High school +1–4 year	583	0.17 (0.08–0.34)	1.15 (0.58–2.22)	99 (12)
High school +>4 years	330	0.19 (0.08–0.40)	1.10 (0.57–2.18)	102 (11)
Maternal age (years)				
<30	443	0.18 (0.08–0.35)	1.15 (0.57–2.24)	99 (12)
30–35	431	0.18 (0.08–0.37)	1.15 (0.57–2.22)	99 (12)
>35	209	0.17 (0.07–0.39)	1.16 (0.67–2.18)	100 (11)
Parity				
1	571	0.18 (0.08–0.36)	1.08 (0.56–2.14)	100 (12)
2 or more	512	0.18 (0.09–0.36)	1.24 (0.61–2.27)	99 (12)
Child sex	Child characteristics			
Boys	616	0.21 (0.09–0.38)	1.21 (0.61–2.27)	98 (12)
Girls	467	0.15 (0.06–0.34)	1.06 (0.55–2.13)	101 (11)
Preterm (<37 weeks)				
No	1036	0.18 (0.08–0.36)	1.15 (0.58–2.21)	100 (12)
Yes	42	0.14 (0.09–0.34)	1.36 (0.58–2.66)	98 (10)
Breastfeeding (months)				
0	38	0.20 (0.09–0.40)	1.09 (0.54–1.88)	98 (14)
0–6	307	0.18 (0.08–0.34)	1.12 (0.56–2.01)	98 (12)
6–12	409	0.17 (0.08–0.35)	1.16 (0.62–2.28)	101 (12)
>12	105	0.18 (0.08–0.32)	0.91 (0.47–2.22)	101 (11)
Missing	224	0.20 (0.08–0.43)	1.24 (0.58–2.51)	98 (12)
How frequently does the child consume organic fruits and vegetables?				
Almost never organic	249	0.21 (0.09–0.40)	1.22 (0.62–2.22)	97 (12)
Half organic	150	0.18 (0.09–0.34)	1.18 (0.66–2.57)	99 (12)
Mostly organic	491	0.15 (0.07–0.34)	1.06 (0.52–2.07)	101 (12)

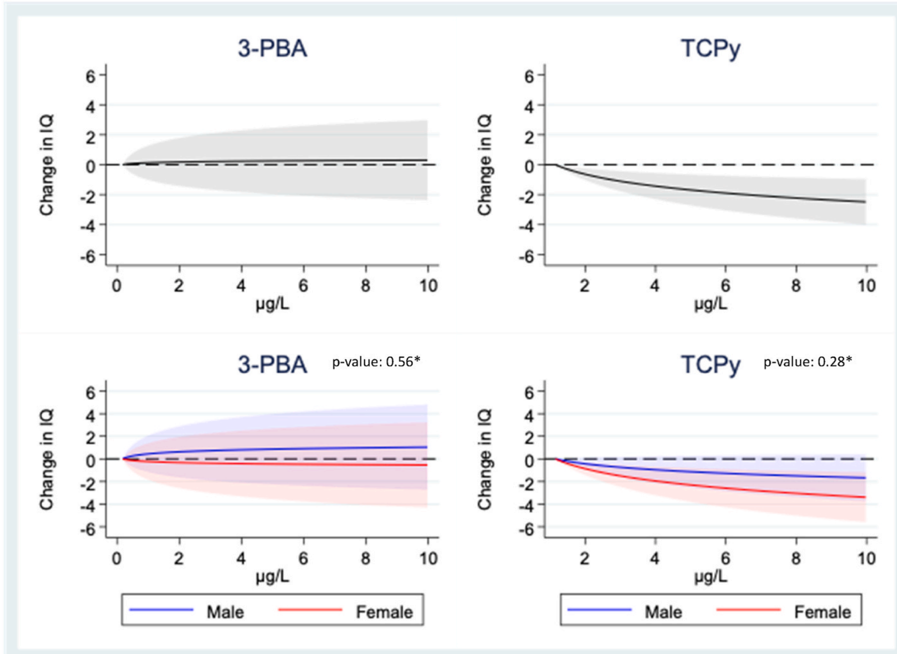

Fig. 2. Estimated differences in intelligence quotient by urinary 3-PBA and TCPy concentrations measured at age 5 years of age and IQ at 7 years of age, adjusted for sex, creatinine and highest achieved maternal education level (as presented in Table 2). The x-axels indicate urinary insecticides concentration. The y-axel represent change estimate in IQ. The dashed lines represent no difference, the solid lines represent point estimates, shaded areas 95% confidence intervals. The top row shows the overall effect, and the bottom row stratified results for sex. IQ: Intelligence quotient, 3-PBA: 3-phenoxy-benzoic acid (pyrethroid exposure) TCPy: 3,5,6-trichloro-2-pyridinol (chlorpyrifos/chlorpyrifos-methyl exposure). *p-value represents the sex differences.

Table 2
Associations between continuous child urinary concentrations of insecticide metabolites at 5 years and IQ and Verbal Comprehension Index (VCI)-score at 7 years. β represent changes in IQ-scores or change in VCI-score (and 95% confidence intervals) for a doubling in urinary concentration among 1083 mother/child-pairs in the Odense Child Cohort.

	β (95 % CI)			
	Full-scale IQ			
	All (crude)	All (adjusted)[a]	Females[a]	Males[a]
N	1083	1083	467	616
3-PBA (μg/L)	0.02 (−0.45, 0.49)	0.05 (−0.41, 0.51)	−0.09 (−0.74, 0.56)	0.18 (−0.47, 0.83)
TCPy (μg/L)	−0.85 (−1.34, −0.35)	−0.80 (−1.29, −0.31)	−1.09 (−1.80, −0.38)	−0.54 (−1.21, 0.14)
	VCI-score			
	All (crude)	All (adjusted)[a]	Females[a]	Males[a]
3-PBA (μg/L) Continuous	0.14 (−0.34, 0.61)	0.16 (−0.31, 0.63)	−0.01 (−0.67, 0.65)	0.31 (−0.35, 0.97)
TCPy (μg/L) Continuous	−0.83 (−1.33, 0.32)	−0.78 (−1.27, −0.28)	−0.77 (−1.50, −0.05)	−0.76 (−1.45, −0.08)

All analyses were adjusted for child urinary creatinine.
[a] Adjusted for: maternal education level and child sex.

Primary analyses were based on a linearity assumption. We performed a sensitivity analysis for the association between exposure and IQ, in which we categorized exposure concentrations into tertiles (Suppl. Table 2). To further assess the potential impact of variables on IQ we also added parity and breastfeeding as covariates (Suppl. Table 3) into our adjusted models to test if this reduced the residual variance.

P-values <0.05 were considered statistically significant. All data were analysed using STATA/BE version 18.0 (StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC.).

2.6. Ethical considerations

The study was performed in accordance with the second Helsinki Declaration, with written, informed consent, and approved by The Regional Committees on Health Research Ethics for Southern Denmark (project-ID S- 20090130) and the Danish Data Protection Agency (13/14088).

3. Results

A total of 1083 mother/child pairs were included. We found no major differences in characteristics between included and excluded mother-child pairs in the Odense Child Cohort (Suppl. Table 1). The majority of mothers had completed high school with 1–4 years of additional education and were younger than 30 years old when they gave birth. The majority of the included children were breastfed for 6–12 months. The overall mean IQ for the children was 100 with a SD on 15. Children of mothers with higher levels of education had higher IQ-scores. Higher IQ-scores were found in girls, and among children with longer duration of breastfeeding, and higher consumption of organic vegetables and fruit.

In the 5-year-old children, 3-PBA and TCPy were detectable in 90%

and 85% of the urine samples, respectively. The median urinary concentrations of the insecticide metabolites were 0.18 (μg/L) for 3-PBA and 1.15 (μg/L) for TCPy. Higher concentrations of 3-PBA and TCPy were observed in boys and among children born at term and who almost never consumed organic food. Women with higher education level tended to have children with lower TCPy concentrations (Table 1).

Fig. 2 illustrates the estimated differences in IQ associated with urinary concentrations of 3-PBA and TCPy measured at age 5 and IQ at age 7. The analysis was adjusted for sex, creatinine levels and maternal education level. TCPy concentrations in urine at age 5 years was associated with lower IQ of the children measured at age 7 years. No association between 3-PBA and IQ-scores was found.

In Table 2 an association between decreased IQ scores for each doubling of urinary concentrations of TCPy was found (−0.80 (95%CI: −1.29, −0.31)). The strongest association was observed among girls with a reduction of −1.09 IQ-points (95%CI: −1.80, −0.38). For boys a similar pattern was seen, although statistically not significant (−0.54 (95%CI: −1.21, 0.14)). Higher TCPy exposure was also associated with a lower Verbal Comprehension Index, overall and for both sexes (Table 2). No associations between urinary 3-PBA concentrations at age 5 and IQ were observed, although there was observed a negative point estimate in girls but not significant. We did not observe any association between 3-PBA and Verbal Comprehension Index (Table 2). The sensitivity analysis, where we adjusted for breastfeeding and parity, did not considerably change the results (Suppl. Table 3).

When categorizing exposure into tertiles (Suppl. Table 2), the highest versus lowest tertile of urinary TCPy concentration at age 5 was associated with −3.18 (95%CI: −5.05, −1.30) lower IQ at age 7. When stratified by sex, the effect size was larger for girls (−4.65 (95%CI: −7.45, −1.85) compared to boys (−2.02 (95%CI: −4.54, 0.51)). For urinary concentration of 3-PBA at age 5, a non-significant decrease in IQ at age 7 was observed for the highest tertile (−0.77 (95%CI: −2.68, 1.14)).

4. Discussion

The children in this study had low median urinary concentrations of 3-PBA and TCPy compared to children in several other studies (Andersen et al., 2022). Despite relatively low exposure, urinary concentrations of the chlorpyrifos metabolite TCPy at age 5 years was significantly associated with lower IQ assessed at age 7 years. This association was slightly more pronounced in girls compared to boys. No associations were observed for pyrethroids, i.e., 3-PBA concentrations and IQ. The differences observed for 3-PBA and TCPy in relation to IQ may reflect a higher neurotoxic potency for chlorpyrifos (European Food Safety, 2019) and/or a higher exposure level as reflected by ∼ sixfold higher urinary concentrations of TCPy compared to 3-PBA.

Few studies have measured exposure through biological samples to quantify childhood exposure to these insecticides and examined their effects on cognitive abilities, as measured by full-scale IQ-scores at school age (Ntantu Nkinsa et al., 2023; Wang et al., 2016).

A cross-sectional study of 406 Chinese children aged 3–6 in Nanjing found a significant association between TCPy concentrations and decreased IQ in unadjusted analyses (Wang et al., 2016). We found more consistent significant inverse association between children's exposure to TCPy and IQ. The Chinese study did not perform sex-stratified analysis, preventing gender comparison. Additionally, they found an association between 3-PBA levels and IQ. While our study did not reveal a clear trend after 3-PBA exposure, we did observe a slight negative impact on IQ, particularly among girls.

Previous studies have demonstrated that the effects of insecticides on neurodevelopment may vary between boys and girls (Andersen et al., 2015; Chevrier et al., 2019; Eskenazi et al., 2018; Llop et al., 2013; Ntantu Nkinsa et al., 2023; Van Wendel De Joode et al., 2016). Sex hormones are vital for brain development and function. They influence, among other things, gene expression in the brain by interacting with hormone receptors that regulate the activity of specific genes and impact cognitive functions such as learning, memory, and spatial abilities (Barth et al., 2015; Iqbal et al., 2024). We did, however, not find a significant association between 3-PBA concentrations in children at age 5 and IQ scores at age 7, although a negative point estimate in girls was observed. This finding aligns with a Canadian study of 179 children, which also reported a negative trend between childhood 3-PBA exposure and full-scale IQ (WISC) in girls, with a mean exposure of 0.39 μg/L urinary concentration (Ntantu Nkinsa et al., 2023), which is higher than ours. Furthermore, a more pronounced effect of chlorpyrifos was observed in this Odense Child Cohort study for the girls.

Also animal studies demonstrates impaired learning and memory associated with developmental exposure to pyrethroids and chlorpyrifos (Abreu-Villaca and Levin, 2017). Additionally, several animal studies indicate that changes in cognitive performance following exposure to chlorpyrifos exhibit distinct gender-specific selectivity (Aldridge et al., 2004; Dam et al., 2000; Garcia et al., 2003; Levin et al., 2002; Venerosi et al., 2012).

This study has several strengths, as its prospective design and large sample size. One of the key advantages of our study is the exposure assessment through measurement in biological samples to estimate pesticide exposures. This method is regarded as the gold standard for exposure assessment as it encompasses all exposure routes and accurately reflects the processes of uptake, absorption, and metabolism (Fenske et al., 2005). The comprehensive nature of Odense Child Cohort due to its large participant base, long-term follow-up, extensive data collection from multiple sources, focus on a wide range of factors, and numerous subprojects also allows for the inclusion of most relevant covariates, providing a robust framework for analysis. The use of WISC-V, an established and validated method for IQ measurement is also a strength. To reduce potential residual confounding, adjustments were made for the level of education of the mother and the sex of the child, which are both acknowledged as predictors of IQ.

This study also has limitations. There was a potential risk of selection bias in the study, since the women included in the Odense Child Cohort were generally older, more likely to be non-smokers, and more often nulliparous compared to those who were not included (Kyhl et al., 2015). However, we found no major differences in characteristics between included and excluded mother-child pairs in the Odense Child Cohort. Furthermore, since the participants were unaware of their insecticide exposure and IQ scores at the time of enrolment, and comparisons were made across different concentrations of insecticide exposure, the extent to which they represented the general population is of less significance. The study demonstrates strong internal validity with no apparent issues. Yet, external validity may be limited if the association between pesticide exposure and IQ differs in the broader population, particularly among lower educated mothers who may consume more non-organic food and have children with lower IQs. Another limitation to consider is the accuracy of measuring insecticide exposure. The insecticide metabolites detected in spot urine samples might not reflect the average exposure of the child over time accurately. This imprecision could lead to exposure misclassification and bias towards the null, particularly given the substantial within-subject variability observed in other studies (Attfield et al., 2014). Nevertheless, it is presumed that urine metabolite concentrations are more stable in populations consistently exposed to low concentrations of insecticides from food residues, such as the present study population, compared to populations with frequent pest control usage (Morgan et al., 2016; Wielgomas, 2013). Though, the most compelling evidence supporting the stability of measured pesticide concentrations in spot urine samples is the observed correlation with dietary habits (Table 1), particularly the consumption of organic foods. Dietary habits, as reported through simple frequency questionnaires, typically reflect consistent and long-term behaviors (Cui et al., 2021), but could be prone to social desirability bias. The observed pattern linking organic food consumption to insecticide metabolite concentrations suggests that high exposures to these insecticides are likely to remain stable over time due to persistent dietary practices.

Despite a significant reduction in the use of organophosphates over the past decade and the EU's ban on chlorpyrifos since 2020 other organophosphates are still widely used in the EU. It is worth noting that urine analyses in this study were conducted before the chlorpyrifos ban in 2020. However, children exhibited lower TCPy concentration in their urine samples compared to their mothers' concentrations collected between 2010 and 2012 (Normann et al., 2024). Future studies should enhance the accuracy and reliability of exposure assessment, as it is crucial to emphasize the need for repeated sampling and a comprehensive understanding of exposure sources. Repeated sampling can help address potential limitations such as residual confounding and improve the precision of exposure measurements. By collecting multiple samples over time, researchers can better capture variations in exposure concentrations and reduce the impact of short-term variations. Furthermore, it would be beneficial to investigate whether these effects are permanent by following the children through puberty.

It is reassuring that we did not find any significant association between 3-PBA and IQ. However, child 3-PBA concentrations in our cohort are very low and fairly uniform, hampering the possibility to detect a dose-response relationship. Thus, studies with a broader exposure gradient or pooling/merging of suitable data sets are needed to identify at which exposure levels these insecticides exert neurotoxic effects. This issue is of major importance since the use of pyrethroids is increasing. Given the ubiquitous exposure to these insecticides, even minor reductions in cognitive development pose significant public health concerns. The cognitive deficits associated with exposure to endocrine-disrupting chemicals are estimated to cost EU countries billions of euros annually, and these costs are preventable (Jones and Schneider, 2006).

5. Conclusion

Our study adds to the growing body of evidence suggesting that childhood exposure to certain insecticides, particularly chlorpyrifos, even at low concentrations, may adversely affect IQ. While most previous studies have focused on prenatal exposure, this study suggests that childhood exposure may also have adverse influence on neurocognition, even at low levels as observed in this study. Although exposure to chlorpyrifos has likely decreased following the EU ban, other organophosphates are still in use and may have similar effects. Whether a switch in use to pyrethroids is a bearable solution remains uncertain, as increased exposure to these compounds could potentially reach levels where they also exert adverse effects.

Future studies should improve exposure assessment accuracy by emphasizing repeated sampling and understanding exposure sources. This approach can address residual confounding, enhance measurement precision, and capture variations in exposure over time.

CRediT authorship contribution statement

Stine Søgaard Normann: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation, Conceptualization. **Helle Raun Andersen:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Lars Christian Lund:** Writing – review & editing, Supervision, Software, Conceptualization. **Iben Have Beck:** Writing – review & editing, Supervision. **Flemming Nielsen:** Writing – review & editing, Validation, Methodology, Formal analysis. **Niels Bilenberg:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Christel Nielsen:** Writing – review & editing. **Þórhallur Ingi Halldórsson:** Writing – review & editing, Supervision. **Tina Kold Jensen:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.120853.

Data availability

The data that has been used is confidential.

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